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FINAL REPORT

NASA CR-

151744Date: 6/5/78Subject: ASSESSMENT OF PANCREAS CELLS NAS 9-15365Part 1. Dispersion, culture, viability and electrophoretic mobility.Part 2. Temperature sensitivity, insulin content and/or production and cellular morphology.Part 3. Ground-based simulation of cell fractionation by preparative electrophoresis.ReferencesFiguresPart 1. Dispersion, culture, viability and electrophoretic mobility.Adult guinea pig pancreas

Pancreatic islets have been obtained routinely from adult guinea pig pancreas, using the collagenase method of Goldman and Colle (1). These pancreas are first cleaned of fat, connective tissue and large vessels, washed in Hanks' Balanced Salt Solution (HBSS) and then minced to about 4 mm<sup>2</sup> pieces. These are shaken gently at 37°C for 60 minutes, in a small tube containing 2.5 ml of HBSS and collagenase (10 mg/ml). After digestion has taken place, the suspension is centrifuged at 30 x g and the supernatant (containing collagenase and debris) is discarded. The residue is washed 3 times with HBSS and then suspended in 3 ml Ficoll-Hypaque (density = 1.080). This is overlaid with HBSS and then centrifuged for 5 minutes at 800 x g. The islets are collected at the interface and separated from cellular debris and non-islet cells (which sediment to the bottom) (2). About 150-300 islets per pancreas normally are recovered. These cells/islets are identified according to established histologic procedures (Part 2) and subsequently kept alive in tissue culture prior to further studies.

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The islets are then explanted into 60 mm diameter Petri dishes containing 15 ml CMRL 1066 (+ glutamine and 10% guinea pig serum) and placed in a 37°C CO<sub>2</sub> incubator (1). The medium is changed every four days. Viability of cells maintained in this way is at least 90% as judged by the trypan blue exclusion test. (Briefly, a drop of islet-containing suspension is mixed with a drop of 0.4% trypan blue dissolved in HBSS and the resultant mixture is evaluated immediately by light microscopy). Those cells/islets that take up the dye are judged dead and those that exclude the dye are judged living.

Electrophoretic mobility of cells obtained by collagenase-trypsin dispersion (3) of adult guinea pig pancreas is measured by analytical cell microelectrophoresis (4). A mobility profile (histogram) results, with values ranging from  $-1.30$  to  $-2.78 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$  with a mean of  $-1.99 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$  (see Fig. 1, page 8). (The buffer used in this case was the one that earlier showed the best resolution in preparative electrophoretic levitation (5): ionic strength 0.04, pH 7.3 phosphate buffer).

#### Fetal guinea pig pancreas

Cell suspensions have been obtained from fetal guinea pig pancreas in much the same manner as from adult guinea pigs, with a few differences in methodology. The density gradient step for islet separation is omitted, and a second collagenase digestion, identical to the first is used to yield a suspension suitable for culture.

The cells are kept alive in the same manner as the adult material. Viability of these cells, as judged by the trypan blue exclusion test, is about 75%.

A mobility profile (histogram), constructed from analytical cell microelectrophoresis data is seen in Figure 2, page 9. Mobility values ranged from  $-1.37$  to  $-2.09 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$ , with a mean  $-1.76 \mu\text{m}/\text{sec}/\text{V}/\text{cm}$ . A phosphate buffer, pH 7.3, ionic strength 0.04, was also used in these measurements.

**Part 2.**      Temperature sensitivity (a), insulin content and/or production (b), and cellular morphology (c).

a)      A portion of a collagenase digest of adult guinea pig pancreas was aliquotted into several small tubes after suspension in "freezing" buffer A-2 (developed in an earlier NASA-sponsored project and reported previously (5)). These were frozen by means of liquid  $N_2$  in a controlled rate freezer at a rate of  $-1^\circ C/$  minute to  $-58^\circ C$  and then conserved in liquid  $N_2$  (6). Several tubes were removed from the liquid after about six months and thawed at  $37^\circ C$ . Viability was assessed by the trypan blue exclusion method. The cells were 70-80% viable as judged by this method.

(b)      Insulin content and/or production was not measured because the required materials (including a large equipment item) were not available during the time frame covered by the contract.

(c)      The study of pancreas cell morphology has been pursued exclusively by standard histochemical techniques using light microscopy. A representative collection of cell types and stains may be seen in Figures 3 and 4, page 10.

**Part 3.**      Ground-based simulation of cell fraction by preparative electrophoresis.

Preparative vertical electrophoresis-levitation of dispersed fetal guinea pig pancreas cells was conducted in phosphate buffer containing a heavy water ( $D_2O$ ) gradient.<sup>1</sup> A representative cell distribution obtained by this means is shown in Figure 5, page 11. The faster migrating fractions tended to be enriched in  $\beta$ -cell content. Alpha and delta cells were found to some degree in most fractions.

The electrophoresis procedure parallels one previously published by us in which the separation of populations of human lymphocytes is described (5). A description of the new method, using a  $D_2O$  gradient follows.

PREPARATIVE ELECTROPHORESIS OF CELLS BY ELECTROPHORETIC  
LEVITATION IN A  $D_2O$  GRADIENT

**MATERIALS**

**Cells.**      Guinea pig pancreas cells were obtained by collagenase digestion of fetal guinea pig pancreas (1).

**Buffers.**      Phosphate buffers of low ionic strength ( $\mu = 0.04$ , pH 7.3) were prepared in distilled water or heavy water ( $D_2O$ ; Aldrich Chemical Co., Milwaukee, WI), consisting of 22.5 mg anhydrous  $Na_2HPO_4$ , 22.5 mg  $NaCl$ , 10 mg  $Na_2EDTA$  and 600 mg glucose for a total volume of 20 ml.

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<sup>1</sup>  $D_2O$  based buffers for increased density, or density gradients offer considerable advantages over sucrose, Ficoll or other polymers, in that they neither cause clumping of cells nor alter the osmolarity of the buffers.

Electrophoresis apparatus. A vertical starch gel electrophoresis frame (Buchler, Fort Lee, NJ) was used to support a cylindrical polystyrene electrophoresis tube, 25 cm in length and 0.75 cm I.D., prepared from a disposable pipet (Falcon Plastics, Los Angeles, CA). This tube requires a water jacket of 4.4 cm I.D. for adequate cooling. A refrigerated water bath (LoTentrol 154, Precision Scientific, Chicago, IL) was used for circulating cooled water through the water jacket. Wicks for the electrophoresis tubes consist of plugs of 2% agarose (Fisher Scientific, Rochester, NY), (dissolved in the same buffer as used for the electrophoresis) at the bottom and a filter paper bridge to the anode chamber at the top.

## METHODS

Electrophoresis. After having been provided with an agarose plug at the bottom, the electrophoresis tube is connected to the cooling water bath and chilled to 4°C. At this point about 0.5 ml of buffer ( $\rho = 1.124$ ) prepared in  $D_2O$  is pipetted above the agarose plug. Then,  $7 \times 10^6$  pancreas cells, suspended in 0.2 - 0.5 ml of a mixture of equal parts  $D_2O/H_2O$  buffer is layered on the  $D_2O$  buffer cushion. The rest of the tube is filled using 1 ml layers containing decreasing amounts of  $D_2O$ . This gives a density gradient of 1.072 at the point of sample insertion to 1.020 at the top (anode) of the electrophoresis tube. The wicks are connected immediately and an electric field of 5 V/cm is applied vertically across the column with the anode on top for 1 hour at 4°C. (The current may rise to about 8 mA, but care should be taken not to allow the current level to exceed that value, otherwise a convective plume of cells will form).

Fraction collection. At the end of the electrophoresis a diffuse zone of cells is visible. Samples of 1 ml each are taken from the top down by means of a 9 inch siliconized Pasteur pipet. Sampling is facilitated by the use of the pipet graduations on the electrophoresis tube. After a cell count of each fraction, the cells are transferred to CMRL-1066 containing 10% fetal calf serum and allowed to rest before being stained for evaluation of cell type.

## RESULTS

Twelve fractions were recovered containing a total of almost  $6 \times 10^6$  cells. A histogram showing the cell count distribution is given in Figure 5.  $\beta$ -cells were concentrated most heavily in the anodal fraction. Fraction 10 - 12 did not contain alpha or delta cells, but did contain beta cells and some endocrine cells.

## REFERENCES

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5. Gillman, C.F., *et al.*, *Preparative Biochemistry* 4, 457 (1974).
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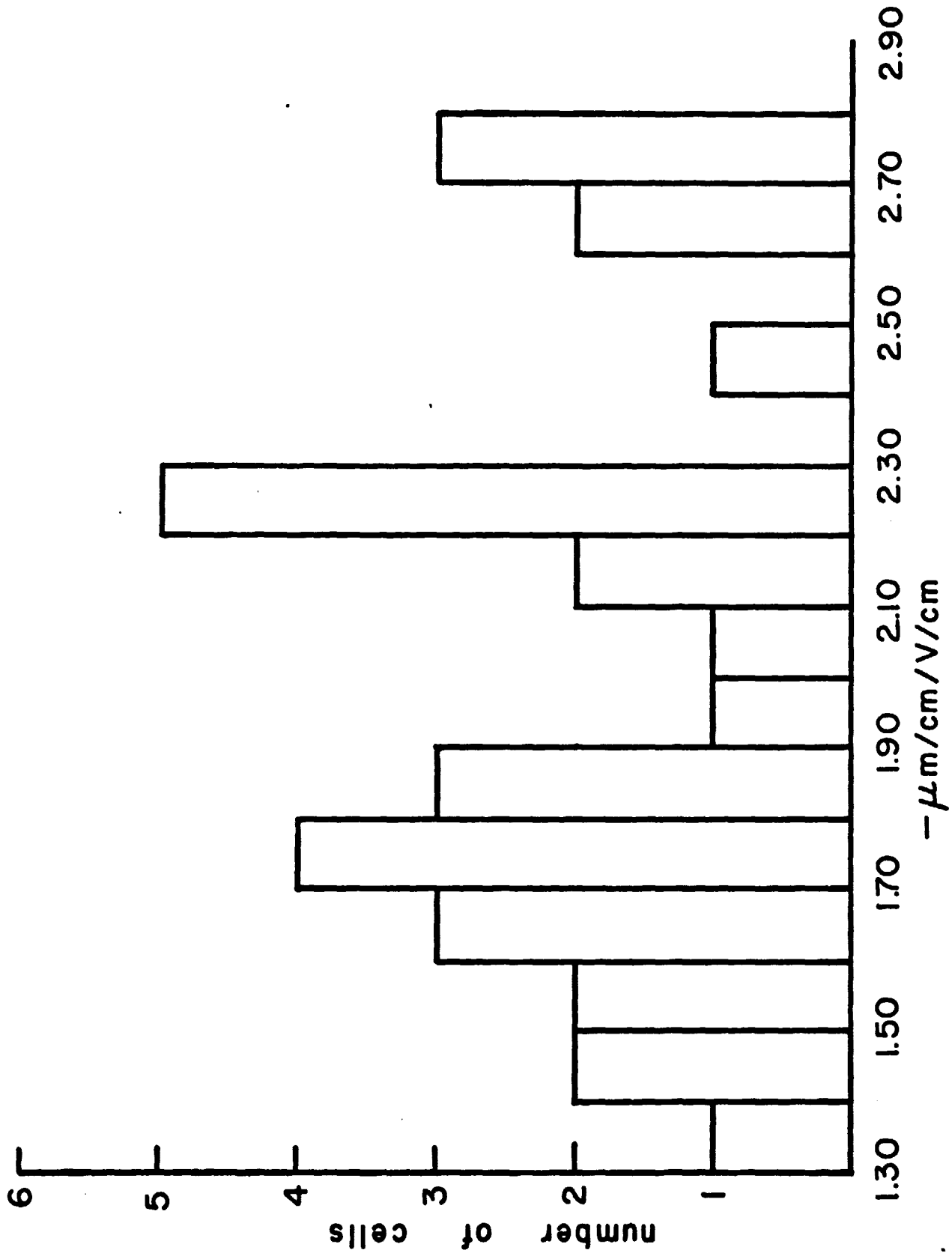


Figure 1. Microelectrophoretic histogram of collagenase-trypsin dispersed guinea pig pancreas cells.  
Phosphate buffer, ionic strength = 0.04, pH 7.3

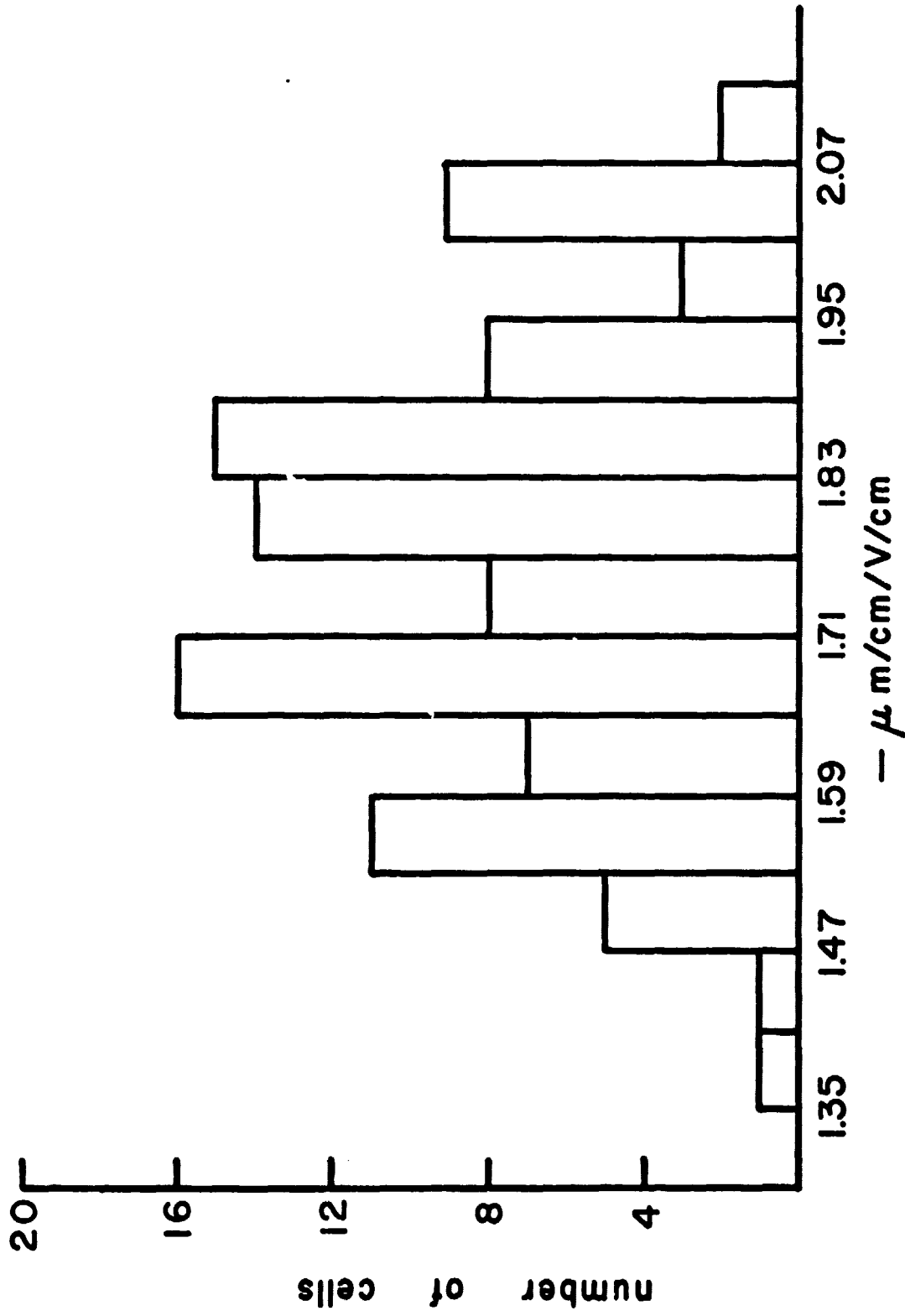


Figure 2. Microelectrophoretic histogram of collagenase dispersed guinea pig pancreas cells ( fetal ).

Phosphate buffer, ionic strength = 0.04, pH 7.3



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Figure 3. Photomicrograph of an adult guinea pig pancreas tissue section showing a large islet, stained by Gomori's aldehyde-fuchsin method. Note the dark-stained granules in the beta cells. The large dark area at the bottom is a staining artifact. Magnification is 100x.

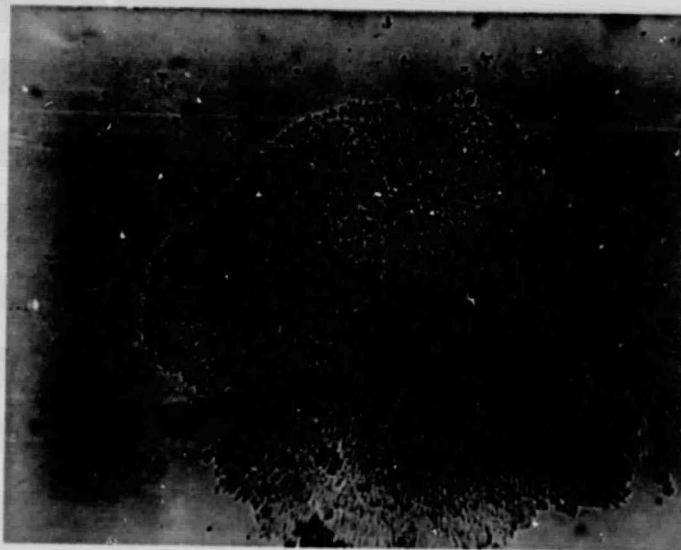


Figure 4. Photomicrograph of an adult guinea pig islet (unstained), isolated, after collagenase treatment of minced pancreas, by centrifugation on Ficoll-Hypaque, density = 1.030. Beta cells are not distinguishable in unstained islets. Magnification is 100x.

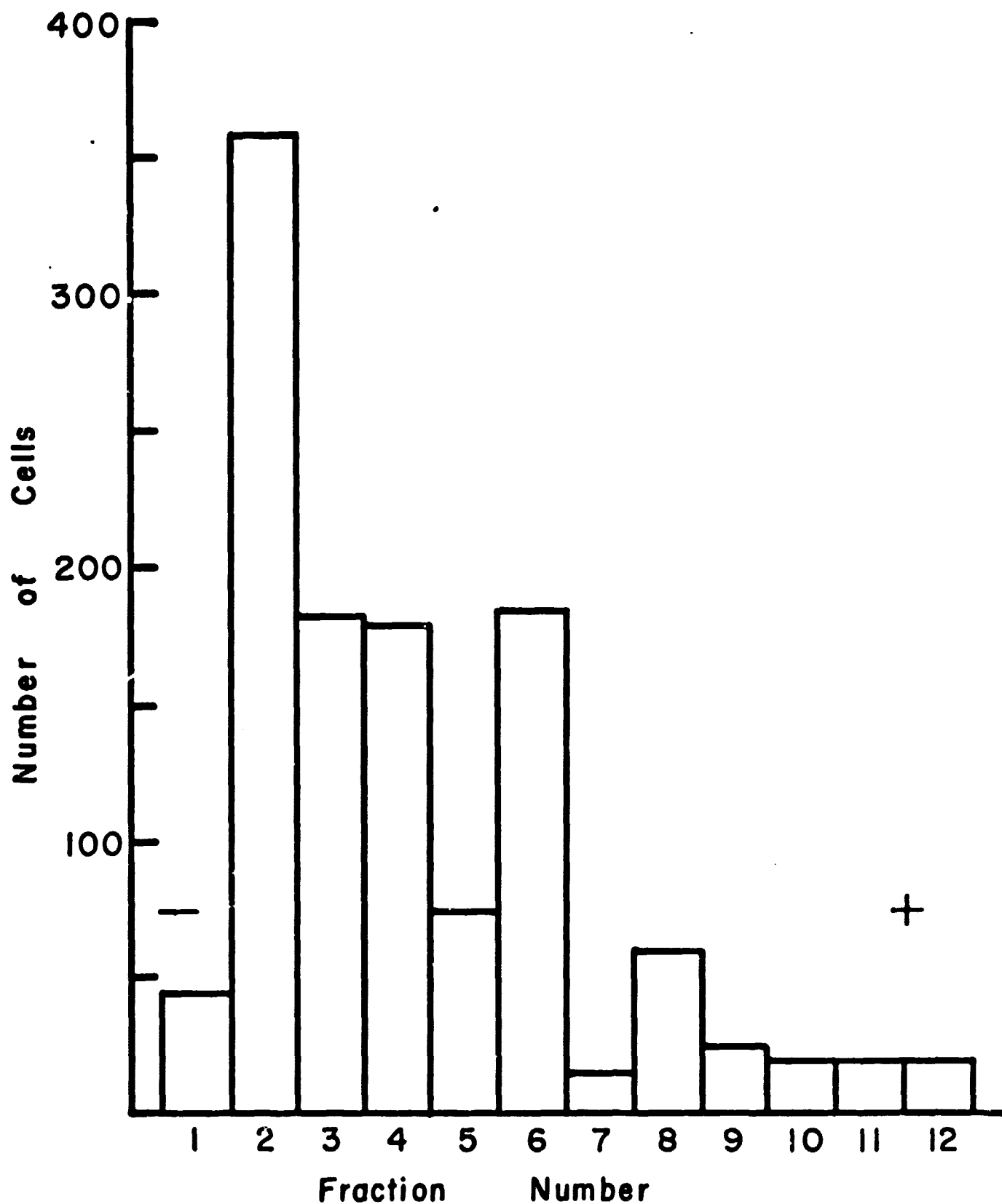


Figure 5. Preparative electrophoresis histogram of  $7 \times 10^6$  guinea pig pancreas cells. Electrophoresis in pH 7.3,  $\mu=0.04$  phosphate buffer. Electric field strength: 5 V/cm. Duration 1 hour. Buffer density 1.072 , fraction 1 ) to 1.020 ( fraction 12 ).